Physiological Relevance and Therapeutic Value of Micro RNA in Cancer

Role of MicroRNA in Cancer

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Abstract

Post transcriptional gene silencing (PTGS) using RNA interference plays an important role against the regulation of parasitic genes. Intensive research during the last few decades on technologies using various interfering RNA molecule to ablate the genes has contributed to new paradigm in the diagnosis and treatment of cancer. It is a promising strategy to inhibit the expression of pathologically relevant genes. PTGS can be mediated either by short or small nuclear RNA (SnRNA) or by micro RNA (miRNA). Among the gene ablation techniques, SnRNA holds superior to the previously demonstrated treatments using antisense oligonucleotides. Expression of miRNA in cancer and their detection by miRNA microarray especially in breast, ovaries, cervix, colon, lung, liver, brain, esophagus, prostate, pancreas, and thyroid cancers have proposed their role in the diagnosis as well as prognosis of cancer treatment. Recently, emerging research in this field adds worth as well as contribution to the development of miRNA based cancer therapy.

Keywords

Micro RNA; Small Nuclear RNA; Gene Therapy; Gene Silencing; Tumor Suppressor Genes; Oncogenes

Introduction

Although substantial progress has been made in the therapy, as well as in the diagnosis of cancer, metastatic neoplasm remains an unbreakable challenge to human kind. Intensive research in the field of cancer during the last few decades has explored the variations in the expression of several genes during the malignant transformation of neoplastic cells. Many of such gene expressions are described under the molecular mechanisms of carcinogenesis as genomic instability. According to theories, cancer can be viewed fundamentally as a genetic disease with alteration of two sets of genes, cellular oncogene and tumor suppressor genes. The alteration of these two sets of genes, irrespective of the etiological factor, has been accepted as unified concept of carcinogenesis. Multiple mutational events that occur in a neoplastic cell during their progression, especially the loss of heterozygosity of most of the tumor suppressor genes, are essential for the malignant transformation of cells. Mutant tumor suppressor's alleles are usually recessive whereas, mutant oncogene alleles are typically dominant.

The extensive use of novel molecular biology techniques in the field of cancer research has identified a large number of molecules involved in the regulation of expression of cellular oncogenes and tumor suppressor genes. Modulation of the activity of these genes at the post transcriptional level referred as post transcriptional gene silencing (PTGS), is achieve by using RNA interference (RNAi) which has an important application in defending the cells against the parasitic genes (Stevenson, 2004). The genes are silenced by RNAi which promotes the degradation of RNA in the cytoplasm. The interference of RNA can be generally modulated using either natural or synthetic oligonucleotides exploiting the Watson-Crick base pairing to target mRNA molecules. Research on the human genome since 2002 supports the diverse and ubiquitous role of small RNA molecules in gene regulation. An antisence therapy using synthetic RNA oligonucleotides that can target and destroy the mRNA, provides outstanding promise for treatment of human disease. However, one of the major obstacles for antisense therapy is efficient delivery of synthetic oligonucleotides to, and uptake into, target cells. Recently, among the natural RNAi, micro RNA (miRNA) mediated regulation ofgene expression has been explored as a diagnostic as well as prognostic measure of diseases. However, their role in the therapy to regulate the expression of the altered gene is yet to be established. This review article discusses the roles of miRNA, and short interfering RNA (Si RNA) in the field of cancer treatment.

RNA Interference

RNAi has an important role in defending cells against parasitic genes by moderating the activity of those gene, which virtually can cause the degradation of any RNA. The previous antisense therapy holds much promise for the treatment of various disorders. Recent RNA based therapy is mediated either by SiRNA or miRNA.

Short Interfering RNA (Si RNA)

Short double-stranded (18-20 RNA bases) RNA molecules induce sequence specific posttranscriptional gene silencing. They can be natural antisense short interfering RNA (nat siRNA) and endoribonuclease-prepared siRNAs (e siRNA). The former is found in the prokaryotes with a definite role and the latter is produced in the cytosol of eukaryotic cells by ds RNA specific endonucleases, Dicer (Hammond et al., 2000). The Dicer enzyme (a RNase III enzyme) catalyzes the production of siRNAs from long dsRNAs and small hairpin RNAs (sh RNA). The structure of SiRNA makes them highly specific to mRNA molecule. The siRNAs in the cytoplasm are separated into single strands and integrated into an active RNA-induced silencing complex (RISC). RISC regulates the target gene by cleaving the complementary mRNA (Fig. 1).

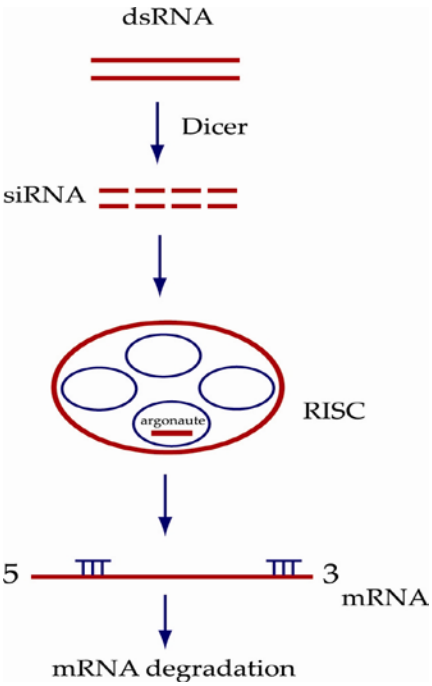

FIG. 1 FORMATION OF SIRNA AND ITS EFFECT ON MRNA

It is the complementarity between the antisense strand of the unwound siRNA and the target mRNA that governs the sequence specificity of gene silencing (Doi et al., 2003). Compared to other gene ablation technologies such as antisense oligonucleotides, siRNAs are superior due to their high degree of specificity, non-immunogenic nature, high resistance to ribonucleases and exclusion from transfer through the nuclear membrane for their activity. Applications of SiRNA include drug discovery, gene therapy, signal transduction and in target validation.

MicroRNAs (miRNAs)

Micro RNAs (miRNA) are small non-coding RNAs (18-25 nts) that regulate a large variety of cellular processes such as cell proliferation, differentiation, programmed cell death, immunity, metabolism and stem cell maintenance (Filipowicz et al., 2008). The first miRNA lin-4, was discovered in *Caenorhabditis elegans* (Lee et al., 1993; Wightman et al., 1993). The human genome consists of more than 1000 genes to synthesize miRNA accounting for about 1- 5% of the total human genes. About 800–1000 miRNAs existing in human, are initially transcribed by pol II as long primary (pri)-miRNAs that are usually capped and polyadenylated. Since miRNAs were recognized in 2001, the broad significance is becoming clear and fully appreciated, because more and more evidence suggest that miRNAs play an essential role in various biological processes.

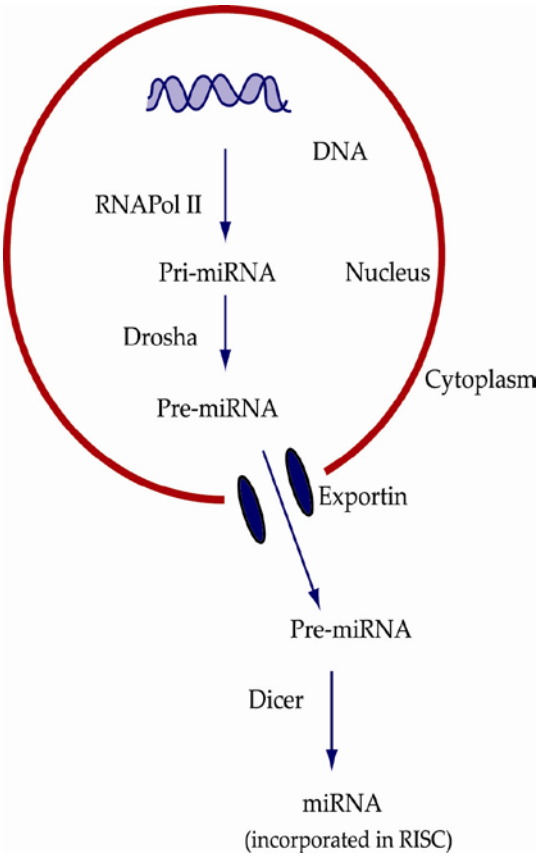

FIG. 2 FORMATION OF MIRNA

The processing of miRNA starts in the nucleus as pri-miRNA. Pri-miRNA is cleaved by a RNase III enzyme, Drosha when it is associated with the binding protein pasha. The cleavage results in the formation of ~70 nucleotide pre-miRNA with a stem-loop structure. Pre-miRNA is transported to the cytoplasm by exportin 5, a transport protein present in the nuclear membrane. The Dicer enzyme further processes the pre-miRNA to miRNA duplex of ~22 nucleotides which is then incorporated to the RISC. In the RISC complex, one strand is retained as the mature miRNA, whereas the other strand is generally degraded (Fig. 2). The translational inhibition depends on the complementarities between the miRNA and the 3′-untranslated region (3′-UTR) of its mRNA target (Winter et al., 2009). Most of the miRNA biding sites in the 3′-UTR lack complex secondary structure, which will facilitate the access to miRNA. Two to eight nucleotides from the 5′ end of the miRNA, called the '*seed region'*, are essential for binding to its target site on a given mRNA. A single miRNAs can bind to 100 different targets.

Physiological Relevance of miRNAs in Human Cancer

miRNAs have been found to have links with some types of cancer, and such miRNA are refered as oncomirs. mirR-21(from *MIR21*) was the first that demonstrated miRNA which is up regulated in wide variety of cancers including breast, ovaries, cervix, colon, lung, liver, brain, esophagus, prostate, pancreas, and thyroid. miR-21 and the miR-17-92 cluster, can also inhibit PTEN expression in human hepatocellular cancer resulting in increased tumor cell proliferation, and invasion (Meng et al., 2007). miRNAs can be up-regulated or down-regulated in various human cancers. Over-expressed miRNAs may function as oncogenes by down regulating tumor-suppressor genes and the down-regulated miRNAs act as tumor-suppressor genes by negatively regulating oncogenes.

Many of the miRNAs modulate the major proliferation pathways through direct interaction with critical regulators such as RAS, PI3K/PTEN or ABL, as well as members of the retinoblastoma pathway (Bueno et al., 2008). PTEN is regulated by miR-214 by directly binding to the *PTEN* 3′-UTR which results in the inhibition of PTEN translation and the subsequent activation of the PI3K/AKT pathway. Hence, expression of miR-214 has been observed in the cell survival and resistance of cisplatin therapy in human ovarian cancer (Yang et al., 2008).

The interaction of miRNA with the regulators of cell cycle such as Cyclin-CDK complexes or cell cycle inhibitors of the INK4 families ($p16^{INK4a}$) or Cip/ Kip ($p21^{Cip1}$ or $p27^{Kip1}$) has been reported (Malumbres and Barbacid, 2001; le Sage et al., 2007). A list of miRNAs that can act as tumor suppressors and tumor promoters is provided in table 1. At least nine members of mammalian let-7 (let-7ai) family of miRNAs negatively regulate *RAS* proto-oncogenes through multiple complementary sites in the 3′-UTRs of all three human *RAS* genes (*H-RAS, K-RAS* and *N-RAS*) (Johnson et al., 2005).

TABLE 1. EXAMPLES OF MIRNA THAT CAN INVOLVE IN THE TUMOR PROMOTION AND SUPPRESSION

Tumor suppressors	**Tumor promoters**
miR-34	miR-17-92
miR-93	miR-21
miR-106b	miR-24
miR-124	miR-106a
miR-126	miR-106b
miR-133	miR-214
miR-137	miR-221
miR-146a	miR-222
miR-203	miR-290
miR-210	

Polymorphisms or Mutations Affecting miRNA

Mutations that result in the polymorphisms affect the functions of miRNA. These will fall into one of the several types

1) ***Mutations Affecting miRNA Biogenesis***

Mutations that affect the processing stages in the formation of miRNA such as the proteins involved in various steps of miRNA biogenesis.

2) ***Polymorphisms in Mature miRNA Sequences***

Polymorphisms in the mature miRNA can potentially influence the expression of many genes

3) ***3Polymorphism/Mutations in miRNA Target Sites***

Single nucleotide polymorphism (SNPs) in the miRNA-binding seed region should disrupt the miRNA-mRNA interaction and affect the expression of miRNA targets which can be sub-classified as 1) at the miRNA binding site and 2) near the miRNA binding site.

4) ***Epigenetic Silencing Of miRNA***

Various miRNA genes are affected by epigenetic

silencing due to aberrant hypermethylation, for instance, silencing of miR-203 by hypermethylation as found in Philadelphia gene positive leukemia. Similarly, CDK6 targeted miR-124 or miR-137 are silenced by hypermethylation in tumor cells of different origins (Kozaki et al., 2008).

Role of miRNA in Cancer Therapies

The results of genomic discoveries suggest incorporating genomic tests or therapeutics into routine care. Manipulation of miRNA-transcription factor gene networks may provide a novel approach for developing cancer therapies. miRNA-based diagnostics and gene therapy are still in their infancy. The induction of RNAi in vivo relies on small interfering RNAs (siRNAs). Instability and poor delivery into target tissues severely limit the therapeutic use of siRNAs. Different approaches including the encapsulation in lipids, complex formation with a variety of liposomes or cationic polymers, chemical conjugation of siRNAs for example to peptides, aptamers or antibodies as well as other formulations have been adopted to favor the cellular uptake, correct intracellular localization and endosomal release. Therapy using miRNA requires the development of efficient delivery systems for siRNA. Synthetic polymers using polyethylenimines (PEI) which are capable to form non-covalent complexes with siRNAs allow their protection from nucleolytic degradation, their efficient cellular uptake through endocytosis and intracellular release. Recently, chemical modifications of PEIs as well as the coupling of cell/tissue-specific ligands are promising approaches to increase the biocompatibility, specificity and efficacy of PEI-based nanoparticles (Günther et al., 2011). However, the biggest problem concerning the use of microRNAs as therapeutics is their pleiotropic mode of action resulting from their wide target of genes present either in the targeted or non-targeted cells.

Conclusion

Mutational inactivation of the tumor suppressor genes and activation of proto-oncogenes are associated with the development of tumor**.** Mutations of *K-RAS*, *APC*, *p53* and *DCC* often occur in tumors**.** Modulation of the activity of genes at the post transcriptional level, post transcriptional gene silencing, is an attractive area to regulate the expression of many genes involved in the malignant transformation of cells. miRNAs, small non-coding RNAs that regulate a large variety of cellular processes, are involved in the regulation of oncogenes and tumor suppressor genes. Ongoing research in cancer will explore the complex interplay between the miRNA and normal or tumoral proliferation which may have relevant implications in not only physiological or developmental processes but also tumor progression or cancer therapy especially preventing cancer development by knockout of oncogene

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